Final Report

Establishment of diagnostic laboratory for mycoplasma diseases of livestock at Ilorin University with particular reference to CBPP and CCPP

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Executive summary

Contagious bovine (CBPP) and caprine pleuropneumonia (CCPP) are OIE-listed diseases because of the socio-economic impact they have mainly on smaller holdings often on marginal land in Asia and Africa. Despite some successful attempts in Nigeria at controlling CBPP in the 1970s there is substantial evidence that the disease is endemic in many parts of the country. CCPP, on the other hand, has been suspected based on clinical and pathological signs but has not been confirmed by laboratory tests. Furthermore, there have been no surveys to show its distribution in the country. Following the purchase of equipment, test kits and reagents and refurbishment and training, a mycoplasma laboratory was established and commissioned in July 2020 by the Vice Chancellor of the University of Ilorin, Kwara state, western Nigeria. A commercial competitive ELISA for the serological detection of the causative agent of CBPP, *Mycoplasma mycoides* subsp *mycoides,* was used to screen abattoirs in the state. Between 6 and 135 samples were taken from each; the percentage of positive sera varied between 0 and 13.5%. However, where more than 40 samples were taken seroprevalence was shown to be between approximately 7 and 14%. Later, 10 cattle were chosen from each of four abattoirs with high seroprevalence for more detailed examination. CBPP-positive cattle (4/10) were found at Ilorin East based on clinical, gross pathological, cultural and serological criterion. *M. m. mycoides* was isolated and identified

using specific staining in diagnostic medium. Cattle from the other three abattoirs were clinically and pathologically negative but some were seropositive and yielded mycoplasmas but these were not considered specific for *M m mycoides*. Confirmation of the Identity of isolates is on-going using growth inhibition tests. In parallel, 10 goats were examined for CCPP at each of these abattoirs. Again, while confirmation is still being carried out on isolates, goats examined at two abattoirs were positive for CCPP based on clinical, pathological and laboratory tests. *M. c. capripneumoniae* was identified based on specific staining in diagnostic medium which represents the first isolation of *M c. capripneumoniae* in Nigeria. Culture-positive goats were also seropositive suggesting CCPP is widespread in Kwara state though number of goats examined was small. A serological survey using the cELISA of over 300 goats kept extensively gave an average seroprevalence of 4.4%. Finally, the cELISA was used to identify various risk factors associated with CBPP infection in cattle herds in Kwara state. Herd size was the most significant factor with herds greater than 100 more likely to have a higher percentage of positive cattle. Evidence was seen that some breeds, White Fulani and Adamwa Gudaly, were more susceptible to CBPP though not significantly. Cattle gender and seasonality were not significantly linked to susceptibility.

Contents

Highlights

- Establishment and commissioning of diagnostic testing for CCPP and CBPP by University of Ilorin
- Identification of causative mycoplasmas of CBPP, *Mycoplasma mycoides* subsp. *mycoides*
- First isolation and identification of causative agent of CCPP, *M. capricolum* susbsp *capripneumoniae,* in Nigeria
- Detection of other ruminant mycoplasmas

- Estimation of seroprevalence of CBPP and CCPP in Kwara state
- Collaboration arranged with Lusaka, Zambia and OIE Reference Laboratory, Gaberone Botswana
- Paper published on previous preliminary studies of CBPP/CCPP

Introduction

Contagious bovine (CBPP) and caprine pleuropneumonia (CCPP) are major pathogens of livestock and listed by the OIE for their socio-economic impact on farming communities. Control of CBPP in Nigeria was nearly achieved in the early 1980s but the disease re-emerged a few years later probably from bordering countries of Niger, Chad and Cameroon (Nicholas et al, 2000). In spite of an eradication campaign launched in 1970, outbreaks rose rapidly from 1986 to a peak in 1989 when over 10,000 cattle were affected. Presently, the disease is widespread in Nigeria (table 1) and the recent wave of civil and religious unrest has enabled the spread of the causative agent, *Mycoplasma mycoides* subsp. *mycoide*s, in the northern part of the country particularly Borno State making accurate monitoring difficult (Olorunshola et al 2017).

Table 1: Officially confirmed CBPP Outbreak in Nigeria (2005-2015)

Year	Susceptible	Cases	Outbreaks	Deaths	Destroyed	Slaughtered
2005	207	22	2	2	0	0
2006	231	65	2	9	0	0
2007	365	707	7	6	0	10
2008	46,919	1,703	28	69	0	0
2009	24,049	1,223	39	72	0	7
2010	9,586	194	17	13	0	7
2011	11,771	316	17	59	7	23
2012	15,345	1,389	6	23	0	0
2013	2,488	158	10	68	0	2
2014	7,500	8	1	1	0	1
2015	7,500	8	1	1	0	1
Total	125,961	5,786	130	323	7	51

Our recent preliminary clinical and serological work using pen-side tests has shown that both CBPP and CCPP are probably present in livestock slaughtered at the abattoirs in Kwara

state (Olorunshola et al 2020). CCPP has been suspected based on clinical and pathological signs but has not been confirmed in Nigeria by laboratory methods. However, the causative agent *M. capricolum* subsp. *capripneumoniae* has been detected by cultural methods in neighbouring countries of Niger and Chad (Manso-Silvan and Thiaucourt 2019) so it is highly likely that there have been incursions of affected goats across borders into northern Nigeria and subsequently into other regions of the country. Furthermore, there have been no surveys to show its distribution in the country. However, identification could be confounded by other respiratory diseases like other bovine and caprine mycoplasmoses so more specific confirmatory testing is required to enable more accurate diagnosis which will enable us to assess the prevalence and economic impact of these diseases locally at first and nationally later.

Aims

The main aim of this project is to set up a diagnostic laboratory for mycoplasma diseases with special reference to CBPP and CCPP at Ilorin University and to determine their prevalence in Kwara State by serology and isolation.

Approaches

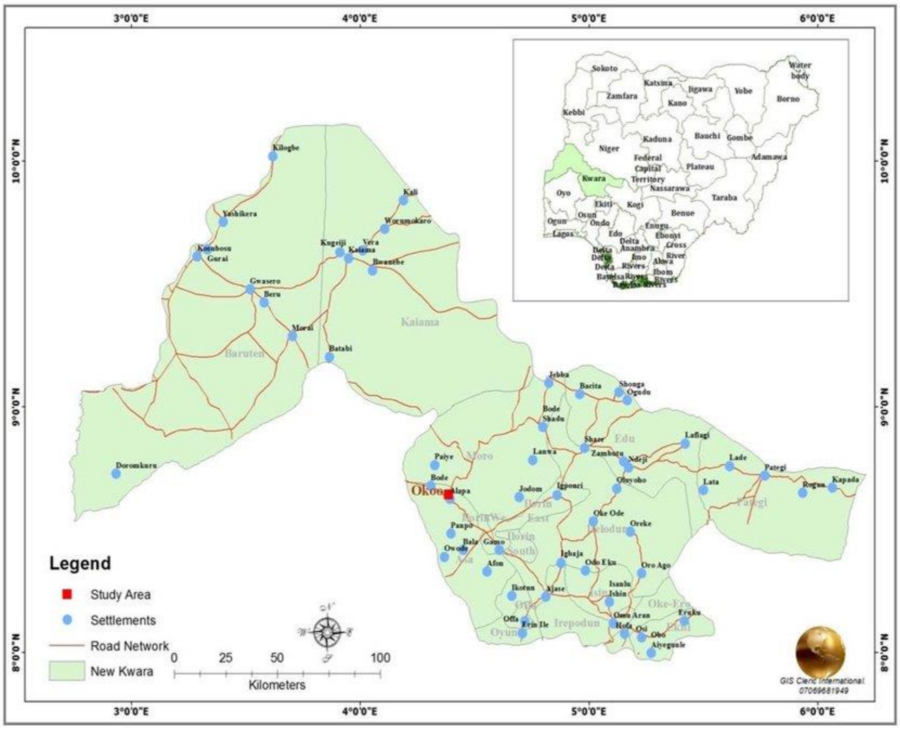
1. Undergo mycoplasma training/discussions at APHA, Weybridge and Mycoplasma Experience Ltd, UK (commercial producers of specialist mycoplasma media and contract testers)
2. Introduce serological testing by buying kits for CBPP and CCPP comprising commercially available latex agglutination (APHA, UK) and competitive ELISA tests (Idexx, France).
3. Establish mycoplasma isolation methods by purchasing commercially available diagnostic medium (Mycoplasma Experience, UK) and to develop and compare home-made media.
4. Obtain specific antisera for commonly occurring ruminant mycoplasmas to enable identification by growth inhibition tests.
5. Initiate molecular identification of mycoplasmas by buying kits and collaborating with other African laboratories
6. Prepare and test bank of serum and clinical samples from cattle and goats at local abattoirs particularly those showing signs of respiratory disease.
7. Investigate reported outbreaks in the field when they occur
8. Explore possibility of twinning with OIE reference laboratory for listed diseases

Materials and Methods

Study Area.

Ilorin is the capital of Kwara state which lies on the plain in the southwest of Nigeria at Latitude 8^{o} 30’ and 8^{o} 50’ N and Longitude 4^{o} 20’ and 4^{o}35’ E. The city occupies an area of about 468 km^{2} situated within the forest and the guinea savannah regions of Nigeria. The Ilorin climate is tropical being influenced by the two prevailing trade winds and experiences an annual rainfall of between from 1000 and 1500mm with daily average temperatures of 25^{o}C, 27^{o}C and 22.5^{o}C in January, May and September respectively. The sampling locations for this study were four local government areas: Moro, Edu, Pategi and Burateen in Kwara state (Fig.1).

Fig. 1: Study area in Kwara State and location in Nigeria



Training

A one-week training period was carried out at the OIE Reference Laboratory for Ruminant Mycoplasmas, APHA, UK covering mycoplasma culture, denaturing gradient gel electrophoresis procedure, Immunoblotting technique, PCR technique, ELISA, DNA extraction and lectures with laboratory experts and a consultant on quality control and standard operation procedures. Contacts were made to facilitate collaboration between APHA and Ilorin University during the project. A full report is attached [Appendix I].

Discussions and advice were given at Mycoplasma Experience ltd UK , specialist media producers and contract researchers. Practical advice was provided and a tour of the facility took place [Appendix II].

Equipment

Essential equipment and reagents were purchased and refurbishment of buildings carried out throughout the project to provide an appropriate environment for this specialized area of bacteriology. A list is included [Appendix III).

Fig. 2 Newly commissioned Mycoplasma laboratory, Ilorin University

Diagnostic tests

(1) Latex agglutination tests which provide rapid serological diagnosis for both CBPP and CCPP were bought from APHA, UK. OIE approved competitive ELISA tests for CBPP and CCPP which provide a more specific and sensitive detection of antibodies were bought from Idexx labs.

Fig. 3 Purchased LAT kits and ELISA reader

(2) While serological tests provide a rapid and sensitive assessment for the presence of antibodies in affected animals it is not possible to distinguish current infection with past exposure so alternative tests are required to look for active infection. Mycoplasma isolation is the gold standard method but can be confounded by mycoplasma fastidiousness and bacterial contamination of the samples. Initially specific media was purchased from Mycoplasma Experience UK Ltd which has the advantage of being quality controlled on low passaged isolates. In addition, while enabling good growth the media also contain a diagnostic property which enables identification of the target mycoplasmas: a red staining of the colonies indicates specific detection on isolated colonies. To confirm these results the growth inhibition test using specific antisera bought from APHA. However, the media are expensive and unsustainable in the long term so home-made media was produced and compared with the commercial product in parallel.

Fig. 4. Growth inhibition tests for mycoplasma identification being performed in safety cabinet

(3) Molecular tests such as PCR are standard techniques in reference laboratories and provide rapid and sensitive detection of specific nucleic acid sequences and overcome the problems of isolation and growth of mycoplasmas but these were beyond the scope of the present project. However, DNA isolations were carried out on a selection of samples and frozen for later in-house identification. In the meantime, some samples were sent to Dr G Muuka at the Central Veterinary Research Institute, Lusaka, Zambia for identification.

Ad hoc investigations of suspected outbreaks

Several field outbreaks suspected to be CBPP and CCPP were investigated

Fig. 5 Extensive goat herds (left) and abattoir (right) in Kwara state

Risk factors associated with CBPP

While plans were being made to sample local abattoirs, serological testing by cELISA of stored and freshly collected sera from suspected herds in the state was carried out looking at various aspects of CBPP infection including: seasonality, prevalence in the local government areas, susceptibility of cattle breeds, different ages and herd size.

Abattoir survey

Four abattoirs were selected for sample collection for CBPP and CCPP in cattle and goats respectively. Sera, lung samples, pleural fluid if present was collected after examining the animals both clinically and pathologically.

Serological survey for CCPP

Goat herds comprising 320 animals in the 4 targeted LGAs were screened with the cELISA for specific antibodies to CCPP.

Results

Establishment of mycoplasma laboratory

Following purchasing of equipment and reagents, refurbishment of the building and training of staff the Mycoplasma Diagnostic and Research Laboratory was commissioned by the University Deputy Vice Chancellor Professor Mikhail Olayinka Buhari for the VC Prof Sulyman Age Abdulkareem on the 9th July 2020 in collaboration with the University of Edinburgh (Fig. 6). The VC promised the continued support of the University.

Fig. 6: Mycoplasma laboratory being commissioned by deputy Vice Chancellor of Ilorin University

Ad hoc investigations of outbreaks

Two nomadic herds in Ovo and Kwara states in the north of the country suspected of being naturally affected herds with CBPP were monitored with serological tests including the latex agglutination and cELISA and examined for specific clinical and pathological signs.

The seroprevalence of CBPP in the 278 head of cattle in Ovo state was over 50% and contained 13% clinically sick cattle while the majority of the 460 cattle herd in Kwara state were morbid with over 66% being seropositive. While slightly less sensitive that the cELISA the LAT was robust and highly effective when used in the field.

Risk factors factors included lack of vaccination, repeated contacts with other herds, lack of veterinary inspection and insufficient feed and water during the dry season.

Long acting tetracyclines, tyrosine and vitamin B complex were applied to the Ovo herd with immediate beneficial results even in cattle overtly sick. In Kwara state cattle were vaccinated and treated with erythromycin and sulphimidine as recommended by sensitivity testing. Recovery again was immediate. It is however very unlikely that herds will be cured of CBPP

Fig. 7: Cow showing respiratory distress prior to post- mortem examination

Table 2: ELISA testing to select abattoirs with most potential cases positives to CBPP

LGA	No of Cattle Sampled	No Positive (%)	OR (95% CI)	p-value
Ilorin West	119	10 (8.4)	0.59 (0.21 – 1.65)	0.40
Patigi	15	0 (0.0)	q	0.34
Ifelodun	6	0 (0.0)	q	1.00
Ilorin East	228	19 (8.3)	0.58 (0.23 – 1.47)	0.29
Ilorin South	52	7 (13.5)		1.00
Offa	70	7 (10.0)	0.71 (0.23 – 2.18)	0.58
Oyun	19	1 (5.3)	0.36 (0.04 – 3.12)	0.67
Asa	28	1 (3.6)	0.24 (0.03 – 2.04)	0.25
Moro	9	1 (11.1)	0.80 (0.09 – 7.45)	1.00
Baruten	44	3 (6.8)	0.47 (0.11 – 1.94)	0.34
Kaiama	48	4 (8.3)	0.58 (0.16 – 2.14)	0.53
Total	**638**	**53 (8.3)**		

LGA: local government areas

Results showed abattoirs in Ilorin west, Ilorin east, Ilorin south and Offa offered best and most convenient opportunity to obtain positive cases (table 2; fig. 8)). In addition, they are the most fully functioning abattoirs and accommodate a larger number of cattle which are brought daily but early attendance is necessary to obtain most rewarding samples

Abattoir survey

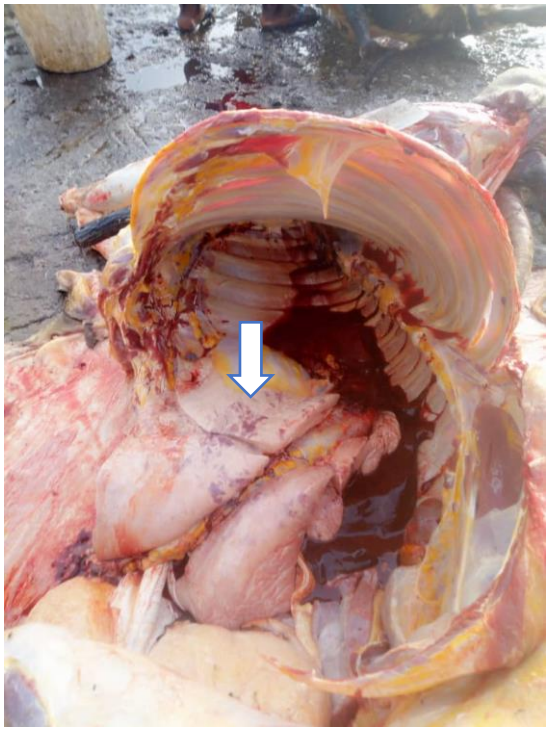
(i) CBPP

Ten cattle were examined for CBPP at 4 slaughterhouses, specifically for typical clinical signs and pathognomic lesions. Blood was taken for serology and lung sampled for culture. Four cattle at Ilorin East showed distinct lesions in the lung (Table 3; Fig 9a). Mycoplasmas were isolated from cattle at all abattoirs but only those at Ilorin East showed specific staining in the ME diagnostic medium (Fig. 10). Growth inhibition tests are being carried out to confirm identity. In addition isolates have been sent to the reference laboratory at Lusaka, Zambia for confirmation. All culture positive cattle were also serologically positive with both ELISA and LAT; however, a single animal was seropositive by LAT but negative by other tests suggesting this could have been a false positive. Other mycoplasmas isolated may have been commensals like *M bovirhinis* or pathogens like *M bovis*; indeed lung lesions in one cow at Ilorin South were very similar to those reported for *M bovis* (fig.9b). Confirmation is being sought.

Fig. 8. Recumbent cow prior to slaughter

Fig 9 (a) Cattle lungs showing fibrin deposition; (b) areas of focal necrosis in the lung (arrowed); (c) pleural fluid in thoracic cavity; (d) sequestrum

(a)



(b)

(c)

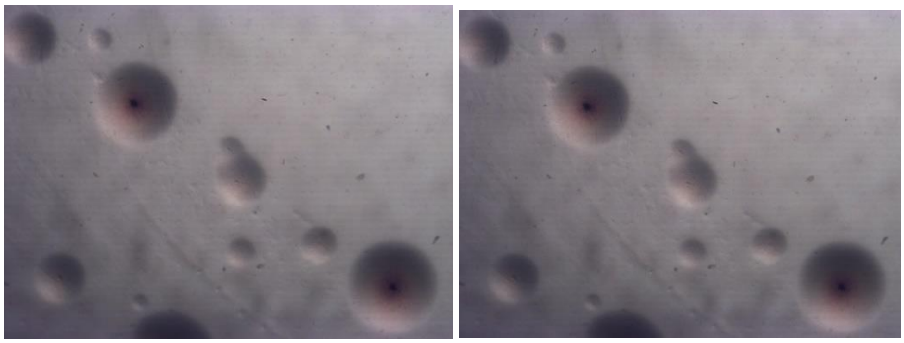
(d)

Table 3: Results summary of cattle pm at 4 abattoirs for CBPP. Abattoir highlighted contain CBPP confirmed cattle based on pathology, culture and serology

LGA	Clinical/pathology signs	Culture	ELISA	LAT
Ilorin South	0	3*	1	0
Ilorin West	0	4*	0	0
Ilorin East	4	3‡	3	4
Offa	0	3*	0	1
	4	**13**	**4**	**5**

* colonies may be commensals like *M bovirhinis* etc
‡ some colonies showing distinct staining for *M. m. mycoides* with ME diagnostic medium

Fig. 10: Mycoplasma colonies showing specific staining indicative of *M. m. mycoides*

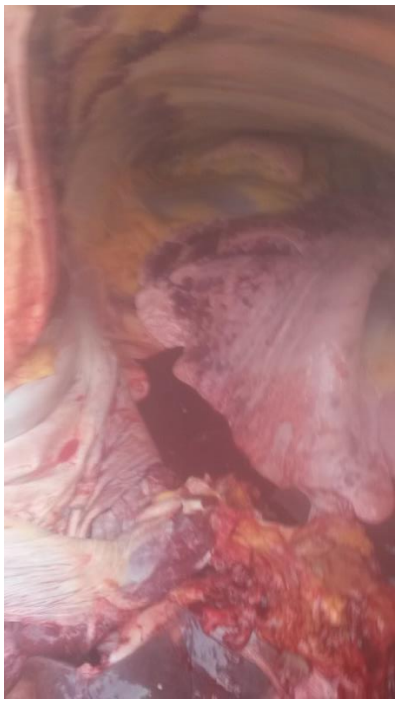


(ii) CCPP

Ten goats were examined for CCPP at 4 slaughterhouses, specifically for typical clinical signs and pathnognomic lesions (table 4). Blood was taken for serology and lung sampled for culture. Goats at 2 abattoirs (West and East) showed lesions suggestive of CCPP (fig. 10) which was confirmed serologically (fig.11). Mycoplasmas were isolated and identified as *M c. capripneumoniae* based on specific staining in ME diagnostic medium (Fig. 12). Further confirmation is being sought as for those from cattle. Other mycoplasmas isolated but not staining may have been *M ovipneumoniae* (fig. 13) or *M arginini*, both of which have been linked to disease in small ruminant. Confirmation is also being sought. Generally, the LAT gave more positive reactions than the cELISA but may have included some false positives due to cross reaction with other members of the *M mycoides* cluster such as *M.m capri* which has a worldwide distribution. The cELISA is more specific and detected those goats from which *M c capripneumiae* was isolated

Fig. 10. Goat lungs showing severe congestion (a), fibrin deposition (b) and pleural fluid containing a lot of blood (c)

(a) **(b)**



(c)

Table 4: Results summary of goat pms at 4 abattoirs for CCPP. Those highlighted contain CCPP confirmed goats based on pathology, culture and serology

LGA	Clinical/pathology signs	Culture	ELISA	LAT
Ilorin South	0	4*	0	2
Ilorin West	4	5‡	2	6
Ilorin East	4	3‡	3	4
Offa	0	3*	0	1
	8	**15**	**5**	**13**

*may be *M arginini* or *M ovipneumoniae* with centreless colonies
‡ some colonies showing distinct staining for *M c capripneumoniae* with ME diagnostic medium

Fig. 11: LAT for CCPP. Left hand card showing negative results; right hand showing positive agglutination

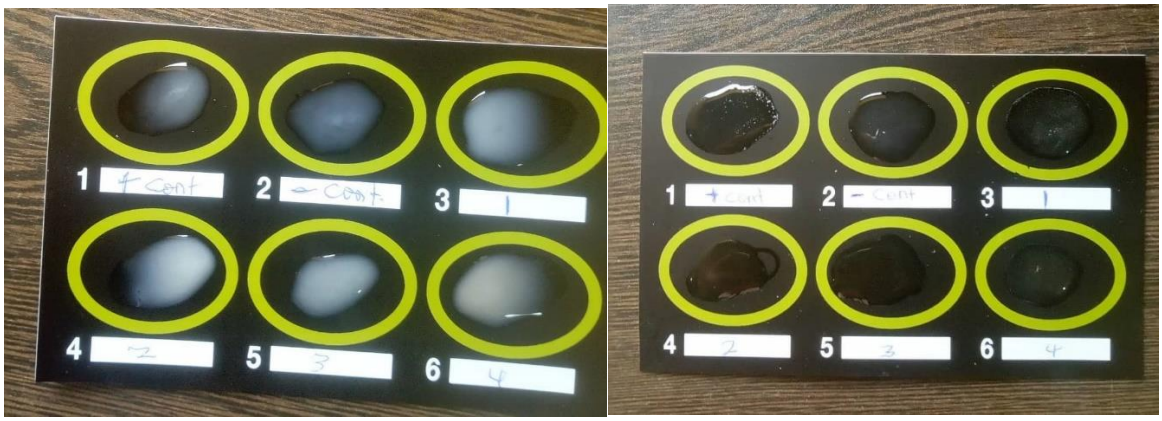


Fig. 12: Mycoplasma colonies showing specific staining indicative of *M. c. capripneumoniae*

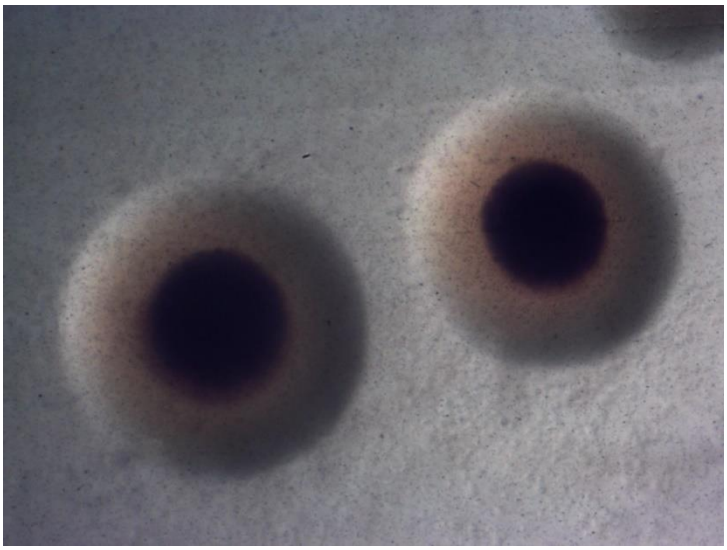


Fig. 13: Centreless mycoplasma colonies indicative of commensal *M ovipneumoniae*

Risk factors associated with CBPP infection

(i) It has previously been observed and reported in our previous study that more females are reared and hence more cows are slaughtered daily. Here we confirm these findings that females are more susceptible to CBPP though not significantly (table 5)

Table 5: ELISA testing of male and female cattle

Sex	No of Cattle Sampled	No Positive (%)	OR (95% CI)	p-value
Male	139	9 (6.5)	1	
Female	499	44 (8.8)	1.40 (0.66 – 2.94)	0.49
Total	**638**	**53 (8.3)**		

(ii) From table 6 it is apparent that more seropositives were found during the rainy season than in the dry season but not significantly

Table 6: Effect of season on detection of seropositives for CBPP

Season	No of Cattle Sampled	No Positive (%)	OR (95% CI)	p-value
Dry	244	19 (7.8)		1
Rain	394	34 (8.6)	1.12 (0.62-2.01)	0.77
Total	**638**	**53 (8.3)**		

(iii) Different breeds of cattle were tested for CBPP. Red Bororo, White Fulani and Adamawa Gudali breeds showed highest percentage of seroprevalence (table 7)

Table 7: Percentage of seropositivty in different breeds of cattle

Cattle Breed	No of Cattle Sampled	No Positive (%)	OR (95% CI)	p-value
White Fulani breed	347	32 (9.2)	1	
Adamawa Gudali breed	114	10 (8.8)	0.95 (0.45 – 1.99)	1.00
Sokoto Gudali breed	51	4 (7.8)	0.83 (0.28-2.48)	1.00
Bokolo	45	3 (6.7)	0.70 (0.21 – 2.40)	0.78
Red Bororo	16	2 (12.5)	1.41 (0.31 – 6.47)	0.65
White Fulani/Adamawa Gudali cross	25	1 (4.0)	0.41 (0.05 – 3.13)	0.71
White Fulani/Sokoto Gudali cross	23	1 (4.3)	0.45 (0.06 – 3.43)	0.71
Sokoto Gudali/Adamawa Gudali breed	9	0 (0.0)	q	1.00
Azawak breed	3	0 (0.0)	q	1.00
Friesian cross breed	5	0 (0.0)	q	1.00
Total	**638**	**53 (8.3)**		

(iv) Slightly higher seroprevalence of CBPP was seen targeting the abattoir rather than farm settlements (Table 8)

Table 8: Comparison of CBPP seroprevalence at different locations

Location	No of Cattle Sampled	No Positive (%)	OR (95% CI)	p-value
Abattoir	338	31 (9.2)	1.28 (0.72-2.26)	0.47
Farm settlement	300	22 (7.3)	1	
Total	**638**	**53 (8.3)**		

(v) Results show the larger the herd the higher the seroprevalence (Table 9) which is in line with studies of *M bovis* and probably indicates greater movement of cattle into the herd (Nicholas et al 2016)

Table 9: Cattle Herd Size for Farm Settlement

Herd size	No of Cattle Sampled	No Positive (%)	OR (95% CI)	p-value
1 - 25	6	0 (0.0)	q	1.00
26 - 50	62	4 (6.5)	1	
51 - 75	169	11 (6.5)	1.01 (0.31 – 3.30)	1.00
76 - 100	44	4 (9.1)	1.45 (0.34 – 6.14)	0.72
>100	19	3 (15.8)	2.72 (0.55 – 13.42)	0.35

Serological survey for CCPP

Goat herds comprising 160 animals in each of the 4 targeted LGAs were screened with the cELISA for specific antibodies to CCPP. Results of the serological screening of goat herds are shown in table 10. In general, seroprevalence was low varying roughly between 1.3 and 7.5% averaging about 4.4% for the state.

Table 10: Summary of the CCPP Screening

LGA	Species	Total sample	ELISA Results	
			No positive	% positive
South	Caprine	80	1	1.3
West	Caprine	80	3	3.8
East	Caprine	80	6	7.5
Offa	Caprine	80	4	7
Total		320	14	4.4

Discussion

Results obtained in this study confirmed the presence of *M m mycoides*, causative agent of CBPP, and showed for the first time the isolation and detection of *M c capripneumoniae*, the causative agent of CCPP, which has long been suspected based on clinical and serological evidence. The use of commercial media and diagnostic tests is probably not sustainable for the mycoplasma laboratory at Ilorin University once funding stops although the University has promised to support the laboratory where it can. However, during the course of the study, successful attempts have been made to produce media for mycoplasma isolation. Preliminary studies have shown that while *M m mycoides* grows well, the more fastidious *M c capripneumoniae* requires improvements to medium formulation. It may also be possible now to produce antigens *in vitro* which can be used for in-house diagnostic tests such an indirect ELISA and LAT.

Both diseases appear widespread in Kwara state and probably throughout Nigeria. In this report a widespread serological survey of 320 commercial goats in the LGAs for CCPP was carried out and showed an approximate seroprevalence of 4.4%. This was lower than the results from a previous study of over 300 small ruminants in Northern Nigeria, (Olorunshola et al, 2020) which used the less specific latex test and could have been cross reacting with a M. mycoides cluster member such as *M m capri* which is widely prevalent. CCPP had a seroprevalence rate in affected herd of about 33% of which about 16% were showing clinical signs. Using the more specific cELISA in our abattoir survey seroprevalence was approximately 12.5% although numbers of animals was much smaller. This still represent a significant level suggesting that CCPP is endemic in parts of Nigeria. Slaughter of these sorts of numbers of herds to control CCPP is clearly prohibitory to owners and authorities who may have to compensate. While antibiotics in particular oxytetracycline and some of the fluoroquinolones are effective against the mycoplasma (Ozdemir et al, 2006) this is an expensive and non- sustainable option for poor farmers and of course can lead to antimicrobial resistance. Commercial vaccines are available against CCPP and have been shown to be protective but are of variable quality (Nicholas and Churchward, 2012). Isolates obtained in this study should be compared with those from other countries to see if any genetic or immunological differences would preclude the use of these commercial vaccines. In the short-term autogenous vaccines could be produced to see if inactivated Nigerian strains protect herds on a small scale. Larger scale trials could then be planned to compare with commercial CCPP vaccines produced elsewhere.

CBPP remains a major problem in Nigeria. In this study seroprevalence was estimated between 8% in a population of over 600 cattle to 10% in our small abattoir survey. It is clear the disease is endemic in many parts of the country and even if successful control was again possible, incursion from infected neighbouring countries would be a continuous problem. So improved border controls are essential but more importantly widespread vaccination aiming to cover at least 80% of the national herd is paramount. This level was achieved 40-50 years ago but the country today is struggling to achieve 20% which is far too small to enable control (Olorunshola et al, 2017). Numerous vaccines have been developed over the last 20-30 years but none have achieved the efficacy of the live T1/44 vaccine which is OIE approved and widely available in Africa. Increased coverage is presently the only solution

with perhaps the use of antibiotics in a targeted manner on herds experiencing severe outbreaks with ring vaccination carried out on adjacent herds.

The newly commissioned mycoplasma laboratory at Ilorin University could play a crucial role in the control of both these diseases in Nigeria as it now has the capacity for both diagnosis and research with enthusiastic and talented leadership but requires both university and government help to sustain its efforts. Formal links with the OIE reference laboratory for CBPP in Botswana and continuing collaboration with CVDRL Zambia will ensure further progress.

Acknowledgements

I want to specially thank our funder – Melinda and Bill Gates as administered by SEBI of the University of Edinburgh under the directorship of Professor Andy Peters. Andy, I am indebted to you for of all your good deeds since I have known you. To my Consultant and Mentor, Dr Robin Nicholas for your able support expressed in expertise and kindness throughout the project. I am indeed very grateful. In the same vein I wish to appreciate Dr Mike Christian-for his advice on this project.

At the outset of this project Robin kindly linked me up with APHA under the headship of Dr Anne Ridley and Helena Windsor, the Director of Mycoplasma Experience limited to observe practice in the field of mycoplasmology. At some points they both supplied the LAT kits, antisera and mycoplasma media that I used in this project. I want to thank my Dean-Professor S.O. Salami who approved a laboratory space for me -that was refurbished and used for this project. He supported me in communicating with the VC and Deputy VC for the approval and commissioning of the laboratory. I want to express my optimum gratitude to the VC-Professor Abdukareem Age and Deputy VC-Professor Mikaili Buhari for the approval and commissioning of the laboratory.

Thanks to my Team, including Dr O.B Daodu, Dr Busayo Ajiboye, Mr Michibi, Mr Akintola, Mr Alada, Usman, Philip, Gbenga, Moses, James and Motun and the current and ex-Directors of the Veterinary Teaching Hospital, the Ambulatory team Dr. Atoyebi, Dr Olowoleni, and Dr Lawal. The Abattoir workers Dr Folaranmi, at Ipata, Akerebiata, Mandate, Ita-Amon and Offa. The entire staff of the faculty cattle ranch and the nomads in charge. I also want to thank my Department including all the academic and laboratory staff, my secretary, office assistant and messenger for their supports. I want to appreciate friends like Koro, Olumide, Taiwo and Ahmed for caring and driving me to the field or office on request. Finally, to my wife and our children for all their supports, and for rendering some critical IT services for pictures and data capturing and collation.

My team

Project leader (left) at University farm

References

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Future plans

Establishment of diagnostic laboratory for mycoplasma diseases of livestock at Ilorin University with particular reference to CBPP and CCPP

General

At the end of this project, Ilorin university will have a functioning mycoplasma laboratory capable of diagnosing mycoplasma infections of livestock in particular the OIE listed diseases CBPP and CCPP, but it should be possible to gain some intelligence on other important diseases such as those caused by *Mycoplasma bovis* which is impacting on all cattle rearing countries. It should also be able to diagnose other OIE diseases causing CA and avian mycoplasmoses

Molecular tests

While some limited identification of mycoplasmas is possible with the help of other laboratories, it will be necessary to bring this in house with the introduction of PCRs for the rapid identification of key pathogens. The establishment of 3-4 PCRs should be sufficient. With these we can detect most 4 small ruminant pathogens including contagious agalactia pathogens. CBPP and *M bovis* Isolates obtained in these studies could also be sent to collaborating labs to see how they compare with other isolates on the continent which may provide information on disease spread

CBPP

Information on the prevalence of CBPP in the state will be known as well as some additional information on breed and age susceptibility, seasonal factors, disease location etc for CBPP. Some knowledge of present vaccination coverage and cattle movements into the area will be valuable to assess control options. However, to improve the influence of the laboratory in the region, it will be necessary to get assistance from OIE laboratories in Africa. Contacts have been made with the lab at Gaborone who are willing to help with identification and other lab activities. I recommend sending Isaac there

CCPP

There are few confirmed reports of the presence of CCPP in Nigeria and we hope to add firm identification to our serological detection by the end of the project. Estimate of prevalence will also guide possible control measures. Commercial vaccines are available but efficacy is variable. They are not presently used in Nigeria because of the lack of awareness of this disease. Vaccine trials either experimental or on farm could be carried out. If successful data will be published then authorities contacted to recommend widespread vaccination rather than the present use of antimicrobials which can lead to resistance. MIC tests on isolates will be useful to assess present risk.

Appendix I

Report of the Training on establishing a diagnostic laboratory for CBPP and CCPP research project in Nigeria
October 14th -18th, 2019

Animal and Plant Health Agency, Addlestone, Weybridge, Surrey, UK

Department of Bacteriology:

Mycoplasma Group

Training overview

The Animal and Plant Health Agency, formerly Veterinary Laboratory Agency is the UK reference laboratory and research institution delegated for the purposes of diagnosis, research and production of vaccines and other biological for animal and plant health. This is also translated into investigating various pathogens and the production of diagnostic kits for the control of disease caused by mollicutes. The goal of this training was for Dr Isaac Olorunshola to acquire some hands on knowledge and skills in the primary isolation of mycoplasmas in ruminants as well as getting familiar with the materials and methods in culture, serology and molecular techniques used in the detection of animal mycoplasmas using modern technology.

Training content

- Registration and security checks at the main gate
- Introduction to the facilities, health and safety procedures
- Specific hand on observation on the following techniques

I. Primary isolation of mycoplasmas by culture:

I observed and participated in the documentation and processing of lung samples submitted from the field with the history of various species of animals suffering from diseases caused by mycoplasmas. Including Poultry, small ruminants, cattle, and others as differentiated and capped with red, white, blue and green covered bijou bottles respectively. Processing was carried out in a sterile biosafety cabinet. Incubation was done using a humidified incubator at 37 degrees with 5% CO_2 for 21 days i.e. 15 working days but growing samples are examined daily for growth or changes involving colour, consistency, turbidity, contamination, all these are recorded and actions taken, like filtration if broth culture are contaminated, sub culturing into fresh broth and sometimes old cultures or irreparable contaminated cultures are discarded. On two occasion's pure broth growth were cultured into PPLO Agar to grow colonies of *Mycoplasma bovis* for me to view using the stereomicroscope-this I observed with pictures of the fried egg colonies taken. Other identification markers are presence of

non-transparent film on top of the mycoplasma bovis broth culture while growing compared with a clean and transparent control broth. Other Mycoplasma species on

broth culture also show a characteristic swirl appearance when shaken first a sort of tornado wind cloud would emerge as opposed to a negative control broth.

II. Other procedures I observed includes:

1. DGGE procedure-Denaturing Gradient Gel Electrophoresis
2. Immunoblotting technique
3. PCR technique
4. ELISA
5. DNA extraction
6. Hours of lectures with laboratory experts and a consultant on quality control and standard operation procedures

The summary of the week-long training programme is as displayed in the Table

SUMMARY TABLE

Date	Anne	Alannah	Owen	Jane	Georgia	Graeme
Monday 14/10/2019	Registration and entry introduction covering health and safety policy	Interactions and asking questions at will	Interactions and asking questions at will	Interactions and asking questions at will	Culture of mycoplasma species	
Tuesday 15/10/2019	Interactions and asking questions at will	Put me through DGGE while Georgia was running it	Interactions and asking questions at will	Put me through the immunobloting to a point but we couldn't finished	Culture of mycoplasma species and examination of growth	Meeting with Isaac quality assurance a laboratory ethical guidance
Wednesday 16/10/2019	Took me through the immunobloting results of different mycoplasma species	Interactions and asking questions at will	Interactions and asking questions at will	Microscopic examination of Mycoplasma mycoides with Isaac	Culture of mycoplasma species and examination of growth	
Thursday 17/10/2019	Together with Isaac visited the ELISA complex. Took me through the immunobloting results of different mycoplasma species	Interactions and asking questions at will	Interactions and asking questions at will	Put me through the immunobloting product from the previous work	Microscopic examination of Mycoplasma mycoides with Isaac And also put me through the PCR	Meeting with Isaac quality assurance a laboratory ethical guidance
Friday 18/10/2019	Microscopic examination of Mycoplasma mycoides with	Microscopic examination of Mycoplasma bovis with Isaac and photography of colonies	Put me through DNA extraction and possibility of paper printing DNA to ease shipment	Microscopic examination of Mycoplasma bovis	Interactions and asking questions at will	

Thanks

I want to specially thank my sponsor, the Supporting Evidence Based Interventions programme (SEBI) of the University of Edinburgh for making it possible for me to undergo this training. To my Consultant and Mentor Dr Robin Nicholas for linking me with APHA the venue of this training. This has enabled me to observe many modern diagnostic techniques gathered more information on various potential mycoplasmas species for research endeavours, I have also expanded my contacts with more experts in this field. Above all the trainng has exposed me to the best practice and quality standards that would make my laboratory and research outputs acceptable internationally following the guides as contained in the ISO and OIE manuals. Finally, I wish to express my profound gratitude to you all, for your care, technical supports to have put me through all your facilities and procedures in spite of your tight schedule am indeed very grateful. There is no amount of words I can offer to commensurate with your kind gesture -I pray that God will continue to enlarge your coast as individual and as a Team. I enjoyed learning from you: Anne, Alannah, Owen, Jane, Georgia, Hilary, Serena, and last but not the least Graeme, as well as my amiable Mentor and Anchor-Robin. May the good Lord bless you all. Bye.

Appendix II

Mycoplasma Experience Visit: The Report

Date =29/10/2019

Venue = Mycoplasma Experience Complex-Redhill –South London

Session A

Discussions on:

Media varieties for CBPP and CCPP, ordering, handling, preservation, transportation, and shelf life. Period of production and validation are critical period of 3 weeks to consider before delivery for use. Helena raised concerns about countries position on importation that I need to clarify shipment permit/license and custom position in Nigeria-paper work on shipment –through courier service providers such as FedEx, DHL, UPS, etc.

Session B

Tour of the laboratory facilities: Such as locations or rooms designated for Cultural isolation efforts, PCR and other molecular assays, isolated rooms for DNA extraction and preservation. Microscopy area for viewing isolates and photography and projections of colonies for proper identification and characterisation. Unit for handling live organisms and strict precautions to prevent cross contamination. I had the opportunity of seeing specialist equipment, materials and consumables for the purposes of media preparation, isolation, storage and preservation of isolates, DNA extraction equipment and kits as well as PCR unit comprising of processing unit-including, loading of DNA/samples, primers, premix and master mixing as well as the computer based interpretations of PCR amplicons.

Session C

Launch break and further discussion: Dave and Helena shared their laboratory experiences with me and responded professionally to all my technical questions on mycoplasma media preparations, isolation and identification techniques. In all, I took note of some vital materials, media, and equipment that I will require and suggested those to Robin-who also witnessed every sessions of this visit. Helena made the photocopies of their media price list for me together with the instructions on how the media could be handled and utilized fruitfully. Both Dave and Helena assured me of their readiness and willingness to address and respond to any challenges that I may be confronted with when am back at work in my laboratory in Nigeria.

Appendix III:

Expenditure: equipment, refurbishment and regents purchased during project

Equipment/ Reagents
ELISA Reader, Kits and Printer
Deep freezer
Standing freezer and fridge
Minor laboratory equipment
Mycoplasma Experience media (2 consignments)
ANTISERA, BIOVILAT AND CAPRILAT reagents from APHA-UK
Avian mycoplasma antigens from Turkey -Free
Routine Laboratory wares and culturing consumables
DNA extraction kits and consumables
Primers for PCR and sequencing
Stereomicroscope and camera
Portable lyophilizer
Single and multi-channelled pipepettes
Laboratory refurbishment
Equipment/ Reagents
ELISA Reader, Kits and Printer
Staff costs

Appendix IV

UNIVERSITY OF ILORIN, ILORIN, NIGERIA

OFFICE OF THE DEPUTY VICE-CHANCELLOR (RESEARCH, TECHNOLOGY & INNOVATION)

DEPUTY VICE-CHANCELLOR (RTI)
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13th July, 2020

Professor Andy Peters
Director,
Supporting Evidence-Based Interventions,
The Royal (Dick) School of Veterinary Studies,
University of Edinburgh, Easter Bush,
Midlothian, EH25 9RG
Ref: ROSLIN 1693

Dear Professor Andy Peters,

Re- Collaboration Agreement between the University of Edinburgh and the University of Ilorin-Letter of Appreciation

I write in respect of the above subject matter. The Administration appreciates the designation of Dr Isaac D. Olorunshola of the Department of Veterinary Microbiology, Faculty of Veterinary Medicine, as the Principal Investigator for the concluded review of diseases of high priority in Nigeria. We also appreciate the recent establishment of a Mycoplasma Diagnostic and Research Laboratory in our University.

The Mycoplasma Laboratory is fully established with modern equipment and standard consumables stocked in the refrigerators. During the lockdown, the Principal Investigator made extra efforts to conduct a trial research on CBPP and CCPP to validate the equipment and ensure that the consumables did not go to waste. The sound results obtained were confirmed by the expert mycoplasmologist (Dr Robin Nicholas) you appointed to supervise the research work in Nigeria.

I am pleased to inform you that the laboratory was commissioned on the 9th of July 2020. Our Veterinary Medicine is relatively a very young Faculty in the University and to have it designated a reference laboratory in this field is no mean feat, given that the only other Reference laboratory for Mycoplasma diagnosis and research is the National Veterinary Research Institute at Vom, Jos, Plateau State. We were also informed that CBPP and CCPP are endemic in Africa and are the main foci of this laboratory. The principal investigator also educated us on this occasion that the laboratory would be useful for internal and external collaborations in animal and human mycoplasmology. It will also be very useful in training, teaching and empowering our undergraduate and postgraduate students. It is our hope the laboratory will attain the OIE reference status soon.

On behalf of the VC, I appreciate the funding of this project. The University support for this collaboration agreement is absolute and we shall continue to provide the necessary infrastructure for the laboratory as our counterpart support. The Principal Investigator has also been advised to apply for additional support from Tetfund to advance the research into Mycoplasmology in Nigeria.
Please, accept the assurances of the highest esteem of the Vice-chancellor.

Yours Faithfully,

Prof. Mikhail Olayinka Buhari